Primary Biliary Cirrhosis is More Severe in Overweight Patients

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Goals: We sought to determine whether features of metabolic syndrome (MS) and histologic features of nonalcoholic steatohepatitis (NASH) are associated with increased fibrosis in patients with primary biliary cirrhosis (PBC).

Backgrounds: PBC is a chronic, progressive cholestatic disease. MS is strongly associated with NASH and fibrosis progression in some liver diseases.

Study: Patients with PBC seen consecutively at the University of Miami between 1985 and 2008 who had antimitochondrial antibody positivity and a liver biopsy performed at this center at the time of diagnosis were identified. Demographics, clinical features, and biochemical parameters were collected. All liver biopsies were reviewed by a single blinded pathologist for features of NASH, PBC, and fibrosis. The impact of NASH and features of MS on liver biopsy findings were analyzed.

Results: Forty-nine patients [median age 51 (34 to 78) years, 98% females] were enrolled. Higher degree of steatosis, severe inflammatory grade, and severe biliary ductal damage were each associated with advanced fibrosis ($P < 0.0001$). Regarding MS, only overweight status [body mass index (BMI) $\geq 25$] was associated with nonalcoholic fatty liver activity score (NAS) $\geq 5$ ($P < 0.0001$), biliary ductal damage ($P < 0.0001$), and advanced fibrosis (71% vs. 32%, $P = 0.007$). Patients with NAS $\geq 5$ had more severe fibrosis (14/15, 96% vs. 11/34, 44%; $P = 0.0001$) and more severe biliary ductal damage (13/15, 87% vs. 3/34, 9%; $P = < 0.0001$).

Conclusions: NASH and BMI $\geq 25$ are associated with severe biliary duct damage and fibrosis in patients with PBC. BMI could become a useful noninvasive tool to predict advanced fibrosis in PBC.

Key Words: primary biliary cirrhosis, metabolic syndrome, nonalcoholic fatty liver disease, steatosis, overweight

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Primary biliary cirrhosis (PBC) is a chronic and progressive cholestatic liver disease that mainly targets the cholangiocytes of the interlobular bile ducts. The etiology of this condition, although believed to be autoimmune in origin, remains unclear.¹ Without treatment, PBC results in destruction of both small and medium sized bile ducts leading to progressive ductopenia and fibrosis, and eventually end-stage liver disease over a period of 10 to 20 years. The disease occurs most frequently in middle-aged women and can ultimately require liver transplantation.²,³,⁴

Metabolic syndrome (MS) is characterized by a constellation of interrelated risk factors for cardiovascular disease and diabetes.⁴ Several definitions have been proposed for MS,⁵⁻¹¹ but in all of them the following criteria are included: hyperglycemia, obesity (particularly central adiposity), elevated blood pressure, hypertriglyceridemia, and low levels of high-density lipoprotein cholesterol.¹² This syndrome is becoming increasingly common in the western world and is estimated to affect over 40 million Americans.¹³,¹⁴ In addition to its association with cardiovascular disease,¹²,¹⁴ MS has been shown to be strongly associated with nonalcoholic fatty liver disease.¹⁵⁻¹⁸

The prevalence of nonalcoholic fatty liver disease correlates with the prevalence of obesity and insulin resistance, whereas the probability of nonalcoholic steatohepatitis (NASH) and more severe stages of liver disease increases with the simultaneous presence of multiple features of MS.¹⁶⁻¹⁹,²⁰ Histologic features of NASH have been reported concomitantly with other forms of chronic liver disease including autoimmune hepatitis and PBC.²¹,²² Components of MS have been recognized as promoting the progression of fibrosis in chronic hepatitis C and alcoholic liver disease.²⁰,²³ Furthermore, the presence of steatosis has been shown to predict more severe liver disease in patients with chronic hepatitis C and in those with hemochromatosis.²³⁻²⁵ Recently, oxidative stress, steatosis, obesity, and alcohol intake were proposed as predictors of histologic progression in PBC.²⁶,²⁷

We hypothesize that the coexistence of one or more components of MS and the presence of histologic features of NASH are associated with more advanced fibrosis in patients with PBC. To address this hypothesis, we conducted a retrospective analysis of patients diagnosed with PBC at the Hepatology Clinic of the University of Miami and evaluated the frequency of MS features and NASH histologic features in these patients and their association with fibrosis stage and biliary ductal damage.

PATIENTS AND METHODS

Study Population

Medical records and liver biopsies from all subjects with the diagnosis of PBC seen at the Hepatology Clinic of the University of Miami from January 1985 to January 2008 were reviewed to select patients with the following inclusion criteria: (1) definite diagnosis of PBC according to

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clinical, histologic, and serologic criteria; (2) liver biopsy performed at our institution at the time of diagnosis and available for review; and (3) reactivity for antimitochondrial antibody (AMA). We decided to include only AMA-positive patients to create a study population as homogeneous as possible. Exclusion criteria included any other form of liver disease including hepatitis B or C, primary sclerosing cholangitis, hemochromatosis, autoimmune hepatitis, overlap syndrome, or alcohol-related liver disease. We also excluded patients with previous use of drugs known to cause NASH (amiodarone, diltiazem, tamoxifen, steroids, and antiretroviral therapy) according to the AGA Technical Review on Nonalcoholic Fatty Liver Disease.

**Histologic and Clinical Data**

Demographics, pertinent clinical features related to PBC and MS, and biochemical parameters at the time of liver biopsy were obtained from laboratory databases and medical records. In this study we adopted the American Association of Clinical Endocrinologists Position Statement on the Insulin Resistance Syndrome to establish criteria for MS. Accordingly, MS was diagnosed in the presence of any 3 of the following criteria: body mass index (BMI) ≥ 25 kg/m²; serum triglycerides ≥ 150 mg/dL; serum high-density lipoprotein cholesterol < 40 mg/dL (in males) and < 50 (in females); blood pressure ≥ 130/85 mm Hg; and fasting plasma glucose ≥ 110 or 120 minutes post-glucose challenge 140 to 200 mg/dL.

All liver biopsies were obtained at diagnosis and follow-up biopsies were not carried out. Patients were naive to ursooxyloxylic acid (UDCA), and were not diagnosed with PBC until confirmation with this liver biopsy. For the purpose of this study, the liver biopsies were reviewed and appraised by a single pathologist, who was blinded to all background information pertaining to the patient’s comorbidities and serologic testing. A biopsy length of > 2 cm and with at least 11 portal tracts was considered adequate. According to our protocol, biopsies were evaluated for grade of steatosis and inflammation, stage of fibrosis, severity of bile duct damage, and ballooning. All biopsy samples were routinely stained with hematoxylin and eosin, silver reelin, Mallory trichrome, Perl Prussian blue, and diastase-resistant, periodic-acid-Schiff stains. The stage of fibrosis was described according to Metavir scoring (0 = no fibrosis, 1 = portal fibrosis without septa, 2 = few septa, 3 = numerous septa without cirrhosis, and 4 = cirrhosis). Steatosis was classified as grade 0 (< 5% hepatocytes affected), grade 1 (5% to 33% hepatocytes affected), grade 2 (> 33% but < 66% hepatocytes affected), and grade 3 (> 66% steatosis). Portal inflammation and biliary duct damage was classified as mild, moderate, and severe if up to 10%, 10% to 50%, or > 50% of portal tracts were involved, respectively. As for lobular inflammation, it was mild, moderate, and severe if 0 to 1, 2 to 4, and > 4 foci per high-power field were involved, respectively. The presence of ballooning, granulomas, Mallory bodies, and bile duct paucity was also evaluated. NASH was diagnosed according to the nonalcoholic fatty liver activity score (NAS) defined as the unweighted sum of scores for: steatosis (< 5% = 0, 5% to 33% = 1, > 33% to 66% = 2, > 66% = 3); lobular inflammation (no foci = 0, < 2 foci per ×200 field = 1, 2 to 4 foci per ×200 field = 2, > 4 foci per ×200 field = 3); ballooning (none = 0, few balloon cells = 1, many cells/prominent ballooning = 2). Cases with NAS of 0 to 2 were largely considered not to be diagnostic of NASH, whereas cases with scores of ≥ 5 were considered diagnostic.

The local Ethics committee approved this study.

**Statistical Analysis**

JMP and SAS (SAS Institute, Cary, NC) were used for statistical analyses. Baseline characteristics were summarized using descriptive statistics. χ² and t test were used for statistical comparisons as appropriate. A 2-tailed P < 0.05 was considered statistically significant.

**RESULTS**

**Baseline Characteristics and Histology**

A total of 49 well-characterized AMA-positive PBC patients [48 females, median age 51 (34 to 78) years] were included. All patients were white, with 14 (29%) being of Hispanic descent. These patients had a median follow-up period of 117 (2 to 293) months. Their clinical, laboratory, and histologic features at the time of diagnosis are shown in Table 1. Except for 3 patients, all were taking UDCA at an average dose of 16.5 mg/kg/d (5.10 to 25.5 mg/kg/d). Three patients had to discontinue UDCA because of worsening pruritus.

Steatosis was verified in 28 (57%) patients, being categorized as grade 1 in 19 (39%) and grade 2 in 9 (18%). Twenty five (51%) patients had advanced fibrosis (stage 3 or 4), 30 (61%) moderate/severe portal inflammation, and 29 (59%) significant portal injury.

**TABLE 1. Clinical, Laboratory and Liver Biopsy Features of PBC Patients at the Time of Diagnosis (n=49)**

| Age at onset* | 51 [34-78] years |
| Female sex | 48 (98%) |
| White | 49 (100%) |
| Hispanic | 14 (29%) |
| BMI* | 25 [18-36] kg/m² |
| Frequency of MS | 10/49 (20%) |
| Features of metabolic syndrome | |
| Overweight (BMI ≥ 25) | 24 (49%) |
| Fasting plasma glucose ≥ 110 mg/dL | 3 (6%) |
| Blood pressure ≥ 130/85 mm Hg | 7 (14%) |
| Dyslipidemia (cholesterol ≥ 200 mg/dL) | 27 (55%) |
| Serum triglycerides (≥ 150 mg/dL) | 10 (20%) |
| Serum HDL cholesterol (< 40 mg/dL in men and < 50 mg/dL in women) | 5 (10%) |

**Laboratory features**

| ALP IU/L (normal: < 165)* | 211 [66-1029] |
| AST IU/L (normal < 40)* | 57 [11-337] |
| ALT IU/L (normal: ≤ 40)* | 62 [24-551] |
| Total Bilirubin (mg/dL)* | 0.7 [0.3-2.2] |
| Albumin g/dL (normal: 3.5-5.0)* | 4.0 [3.2-4.8] |
| INR* | 1 [0.8-1.25] |

**Liver biopsy**

| NAS 0-2 | 18 (36%) |
| NAS 3-4 | 16 (33%) |
| NAS ≥ 5 | 15 (31%) |
| Fibrosis (Metavir F3/F4) | 25 (52%) |
| Biliary duct damage (moderate/severe) | 30 (61%) |

*Median and range.

ALT indicates alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; INR, international normalized ratio; MS, metabolic syndrome; NAS, nonalcoholic fatty liver activity score.
23 (47%) moderate/severe biliary duct damage. The presence of ballooning, Mallory bodies, and granulomas was verified in 42 (86%), 16 (33%), and 26 (53%), respectively. Using NAS score, 18 (32%) of patients scored 0 to 2, 16 (33%) scored 3 to 4, and 15 (31%) scored ≥ 5 (diagnostic of NASH).

**Impact of Histologic Findings of NASH on Fibrosis**

Patients with higher degrees of steatosis were more likely to have advanced liver fibrosis. Only 5/21 (24%) patients with grade 0 steatosis had advanced fibrosis, compared with 11/19 (57%) with grade 1 and 9/9 (100%) with grade 2, P < 0.0001. The presence of moderate/severe portal inflammation and advanced/severe biliary duct damage were also significantly associated with advanced fibrosis (P < 0.0001 and P = 0.0002, respectively). In addition, steatosis was also significantly associated with the degree of portal inflammation (P < 0.0001) and with biliary ductal damage (P = 0.001). Patients with NAS ≥ 5 were more likely to have advanced fibrosis (14/15, 96% vs. 11/34, 44%; P = 0.001) and to have more severe biliary duct damage (13/15, 87% vs. 3/34, 9%; P < 0.0001) as shown in Figure 1.

**Impact of Features of MS on Fibrosis**

Overweight status (BMI ≥ 25) was the only feature of MS associated with advanced fibrosis (17/24, 71% in patients with BMI ≥ 25 vs. 8/25, 32% in patients with BMI < 25, P = 0.007). Overweight status was also associated with NAS ≥ 5 (23/25 (92%) versus 11/34 (34%), P < 0.0001 and moderate/severe biliary ductal damage 15/24 (62.5%) versus 1/25 (4%), P < 0.0001, as shown in Figure 2.

Other features of MS (diabetes mellitus, hypertension dyslipidemia) taken individually were not associated with advanced fibrosis or degree of steatosis. A trend towards more advanced fibrosis was observed in patients with more features of MS, but this was not statistically significant: 6/20 (30%) patients with no features, 10/18 (55%) patients with 1 feature, 3/3 (100%) patients with 2 features, 5/7 (71%) patients with 3 features, and 1/1 (100%) patient with 4 features of MS had advanced fibrosis (P = 0.07). Age, sex, hepatic biochemistries, and the presence of ballooning and granuloma on liver biopsy were also analyzed and were not associated with steatosis, NAS, or fibrosis (data not shown).

**DISCUSSION**

The current study reports a significant association of NASH and overweight status with advanced fibrosis in patients with PBC. These 2 variables were also associated with the presence of severe portal inflammation and severe ductal biliary damage. Our findings suggest that clinical and histologic features of fatty liver disease are of clinical relevance and may negatively impact on disease progression in patients with PBC.

The concurrence of clinical and histologic features of NASH with other chronic liver diseases has been recently reported and may be a reflection of a worldwide increase in the prevalence of NASH. Steatosis has been reported in >50% of patients with hepatitis C infection, being related to obesity and NASH in those infected with genotype 1 but not in their counterparts with genotype 3, where viral factors have been implicated. Furthermore, steatosis was found to be independently associated with stage of fibrosis in both hepatitis C and hepatitis B infections, and with the annual rate of fibrosis progression in chronic hepatitis C infection. The concurrence of steatosis in patients with PBC has also been described, although its impact on disease progression needs further clarification.

A clinicopathologic study evaluating 232 needle liver biopsy specimens from patients fulfilling criteria for PBC described steatosis as being remarkable in 50% (24). More recently, Sorrentino et al. reported steatosis in 40.5% and NASH in 14.9% of a cohort of 274 Italian patients with AMA-positive PBC and verified an independent association between oxidative stress, steatosis, higher BMI and alcohol intake, and more advanced disease stage. Our findings are in agreement with Sorrentino and colleague’s data: overweight status, steatosis, and NASH were common findings in our patient population, with a frequency of 49%, 57%, and 31%, respectively, and those features were associated with advanced fibrosis.

The underlying mechanism through which overweight status and steatosis would lead to faster histologic progression of PBC is subject of debate. Although oxidative stress due to lipid peroxidation has been demonstrated in a subset of patients with PBC and steatosis, it is well recognized that adipose tissue in obese patients develop a more dysfunctional phenotype with proinflammatory, profibrotic, and proangiogenic properties. Furthermore, adipokines and proinflammatory cytokines secreted by visceral adipose tissue easily reach the liver through the portal vein, leading to local vascular effects that may result in worsening of portal hypertension. Very recent evidence that obese patients have more clinical decompenensation of cirrhosis substantiates this hypothesis. In a study including 161 patients with well-compensated cirrhosis, independent predictors of first clinical decompenensation were serum albumin level, hepatic venous pressure gradient (HVPG), and BMI. Moreover, overweight and obese patients had significantly less reduction in HVPG after 1 year of follow-up, despite the fact that half of the patients were receiving a nonselective β-blocker. Thus, it was hypothesized that perhaps the mechanism through which obesity leads to clinical decompenensation would be at least in part caused by an increase in HVPG, which is, in turn, caused by the increased intrahepatic resistance generated by adipokines and cytokines. Interestingly, the present study also noted an association of steatosis and BMI with portal inflammation and biliary ductal damage. Thus, specifically in the case of PBC, it is possible that more severe biliary damage was worsened by this proinflammatory state, leading to increased apoptosis. Apoptotic biliary epithelial cells are known to represent a source of preserved...
immunogenic pyruvate dehydrogenase complex-E2, leading to antigen recognition and destruction of biliary epithelial cells by an activated innate immune response, thus triggering a self-perpetuating cycle that results in induction of fibrogenesis and progression of disease.

Our study is limited by a small sample size. We utilized strict criteria for study entry, which allowed for a homogenous population and eliminated missing data on histology, thus strengthening the results. Furthermore, it has been repeatedly demonstrated that AMA-negative PBC has similar natural history to AMA-positive PBC, therefore, we do not expect that the exclusion of patients with AMA-negative PBC would impact our results. Finally, given the retrospective nature of the study, we did not have sufficient data to assess the impact of BMI and steatosis on the response to UDCA therapy; this should be addressed in future studies. In addition, we also verified a trend towards more advanced fibrosis in patients with more features of MS, although this association did not reach statistical significance. Thus, further evaluation is required to determine whether the presence of MS represents a prognostic marker in PBC.

In conclusion, our observations add to currently available data pointing towards a clinically significant impact of overweight status, steatosis, and NASH in the disease progression of PBC. In particular, BMI ≥ 25 could be useful as a noninvasive tool to assist with decisions regarding the need for liver biopsy and the timing for implementation of surveillance strategies for hepatocellular carcinoma and esophageal varices. Therefore, larger studies are recommended to assess the impact of BMI on the clinical progression and response to therapy in patients with PBC.

REFERENCES